

# **LOCO: Characterization of Phytoplankton in Thin Optical Layers**

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## **LONG-TERM GOALS**

Our long-term goal is to understand the ecology of phytoplankton inhabiting coastal shelves, upwelling areas, fjords and banks. We are especially interested in ways in which species-specific properties, including colony size and shape (diatoms) and motility (dinoflagellates) interact with physical mixing processes to regulate spatio-temporal distribution patterns. We wish to understand these processes in sufficient detail to be able to predict bloom dynamics, size structure, and the impact of species-specific characteristics of the phytoplankton on ocean optics.

## **OBJECTIVES**

Our goals within the LOCO DRI program are (1) to thoroughly characterize the phytoplankton community within thin layers and compare it to that outside of layers, (2) to increase our understanding of the importance of species-specific characteristics of the plankton to both ecology and ocean optics, and (3) to expand our understanding of the role that biological-physical processes play in thin layer dynamics.

## **APPROACH**

Under previous ONR funding (N000149610247, N000140210247), we have demonstrated that interactions between physical processes at multiple time and space scales, and the species-specific properties of diatoms and dinoflagellates (*e.g.* size, shape, behavior etc.) are important factors contributing to phytoplankton distribution, bloom dynamics, particle size structure and optical characteristics in the ocean. In order to continue this work within the LOCO framework, we have (1) adapted our earlier protocols for use in the open waters of Monterey Bay (*i.e.* exposed, coastal locations), and (2) developed methodologies that will allow us to collect new kinds of data, so that we can begin to investigate our ‘next generation’ of questions. In August/September of 2005, and in July 2006, we employed our refined protocols during the LOCO field experiments in Monterey Bay, California. Our primary effort was carried out in close collaboration with Donaghay, Sullivan, Holliday and Hanson, working from *R/V Shana Rae*. We are fortunate to also have the opportunity to collaborate with the many additional PIs in the LOCO program.

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## WORK COMPLETED

Our efforts in FY08 were divided between analysis of samples collected during the 2005 and 2006 LOCO field experiments, preparation of manuscripts, and further evaluation and development of the capabilities of our CytoSense scanning, in-line flow cytometer, which can be used both bench-top and *in situ* - <http://www.cytobuoy.com/>

**LOCO Sample Analysis.** Our sample set from the LOCO experiments includes over 300 preserved whole water samples collected from inside and outside thin layers and surface slicks, about 30 offshore samples from *R/V New Horizon* (2005), live counts (2005) of the fragile dinoflagellate *Akashiwo sanguinea* (which does not reliably preserve), over 100 filter samples for epifluorescence-based image analysis of picoplankton, over 20 hours of videotaped record of microscopic examination of live phytoplankton, and about 70 CytoSense samples (2006). Analysis of samples has been prioritized to address specific questions, and to interact with other members of the LOCO team. We currently have several manuscripts in preparation.

**CytoSense/CytoSub Evaluation.** We conducted a series of experiments designed to evaluate the machine's abilities, and to cross-calibrate CytoSense data with that generated from our other methods of analysis. To assist in this work, an engineer from CytoSense (Woerden, the Netherlands) visited our lab in February, to help fine tune the instrument's hardware and software to best meet our needs. With these refinements in place, we evaluated the instrument's ability to:

- accurately measure cell and colony density for a range of concentrations
- accurately measure cell and colony length for a range of sizes
- accurately quantify the number of cells in a colony
- resolve terminal setae (spines) in members of the species-rich diatom genus *Chaetoceros*
- resolve heavily silicified intercalary setae and intercalary terminal setae (evidence of incipient colony division) in species of the genus *Chaetoceros*
- resolve colony curvature
- identify dead cells within a colony
- provide sufficient information to discriminate species or species groups

## RESULTS

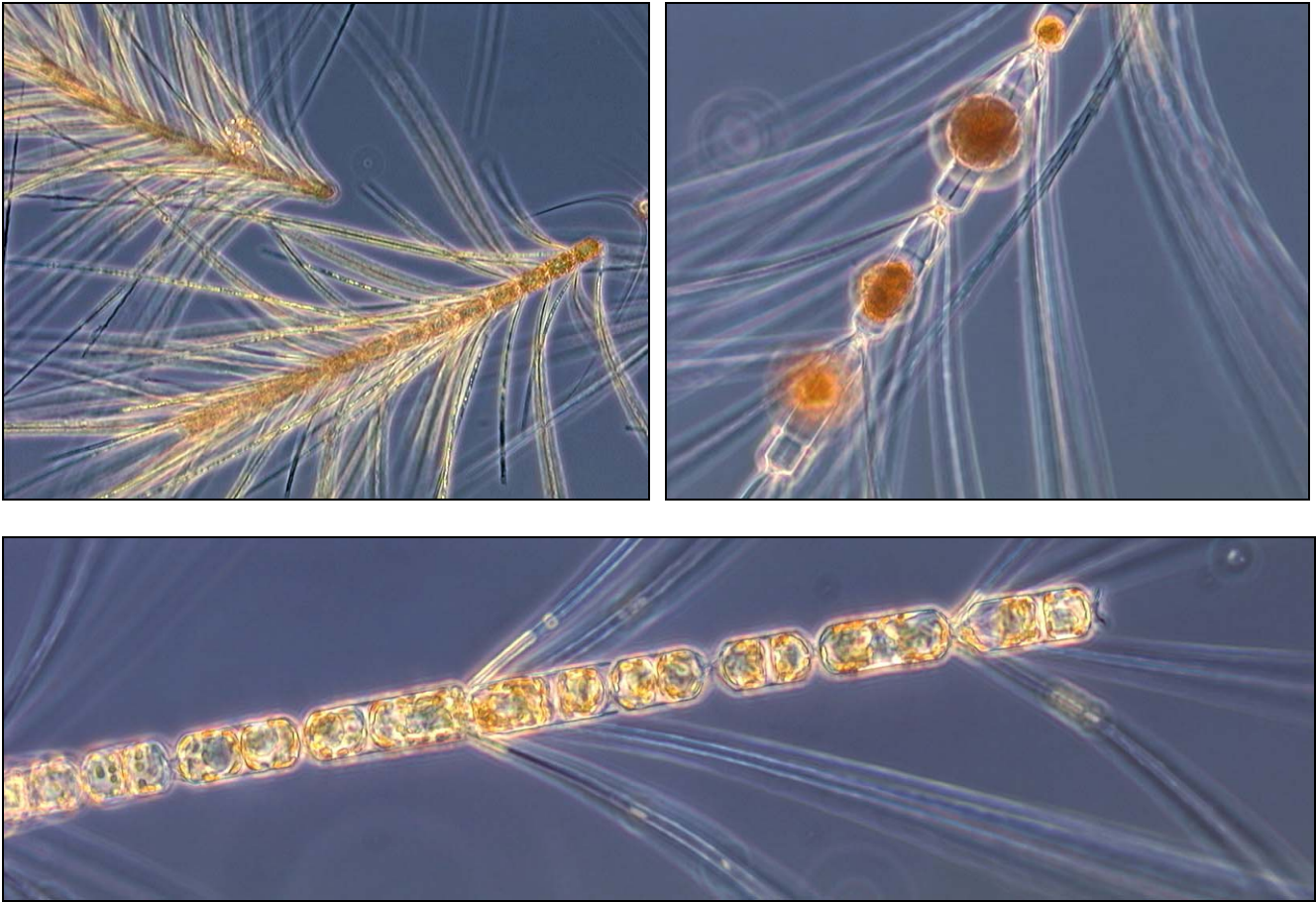
**LOCO.** Monterey Bay is home to an extraordinarily diverse community of phytoplankton. Although many of the same taxa were present in both 2005 and 2006, the dynamics of each year were quite different, and were correlated to hydrographic patterns. Our extensive data set, coupled with those of our colleagues, allows us to address many of our originally proposed questions, including:

- Are thin layers composed of an enhanced concentration of the total integrated phytoplankton community, or are they dominated by a particular taxon, or size/shape class?
- Are spatially extensive layers taxonomically uniform?
- Are all taxa and size classes of phytoplankton capable of forming layers?
- Are motile phytoplankton more likely to form layers?
- Are layers/regions of low turbulence a refuge, which permit the development of a large population of diatoms of complex shape?

Once characterized, the implications of these features of the water column can be explored. Thin layers are of interest for multiple reasons, including their potential impact on both the oceanographic environment, and Navy sensor systems. For example, thin layers affect the inherent optical properties (IOPs) of the water column (Sullivan *et al.* 2005, Sullivan *et al.* submitted), and because they offer dense concentrations of food potentially attractive to zooplankton and planktivorous fish (acoustical scatterers), they also impact ocean acoustics (Holiday *et al.* 2003, Holliday & Stanton 2005).

But thin layers are also interesting because they may be critical to the organisms themselves. Many ecological processes are concentration dependent, whereby a certain threshold must be reached in order for a specific process to take place. The threshold value (or window, if there is a second, upper threshold above which no additional benefit is accrued) can be considered the scale of critical importance to the organism. For example, a critical concentration of appropriate food must be present to ensure the success/survival of a cohort of fish larvae. Dense patches and thin layers of phytoplankton such as the thin layer of the dinoflagellate *Akashiwo sanguinea* present during our 2005 experiments can offer the requisite source (*e.g.* Lasker 1975, Kiefer & Lasker 1975, Lasker & Zweifel 1978). Therefore, from the perspective of the organism, thin layers can be viewed as *critical scale* phenomena, which may be essential to their ecological success.

Another example of the biological importance of thin layers is found in our 2006 Monterey Bay data. On July 23<sup>rd</sup> we observed a thin layer of the colonial diatom *Chaetoceros concavicornis* at about 11 meters depth (Rines *et al.*, *in prep.*). This taxon is implicated in harmful algal blooms (HABs) because its serrated, siliceous setae (spines) lacerate the gills of salmon restrained in fish farm pens, resulting in mortality and economic loss. Concentrations as low as 5 cells·ml<sup>-1</sup> can prove lethal. Concentrations in our Monterey Bay thin layer exceeded 200 cells·ml<sup>-1</sup>! Thus, the dense concentrations of an inimical organism comprising a thin layer are relevant both to the impact of the HAB, and to our ability to detect it, as previously discussed by Rines *et al.* (2002). Additionally, formation of a thin layer appears ecologically relevant to the *Ch. concavicornis* population itself. Diatoms undergo prolonged periods of asexual, mitotic division, resulting in large populations of vegetative cells. Periodically, they undergo sexual reproduction. An obvious challenge to this process is that compatible gametes must be successful in finding each other in a dilute, watery environment. We have hypothesized that the close proximity of cells in a dense thin layer should facilitate this process. Consistent with these ideas, male gamete formation, as well as auxospores (the successful product of sexual reproduction) were observed in this population (Figure 1).



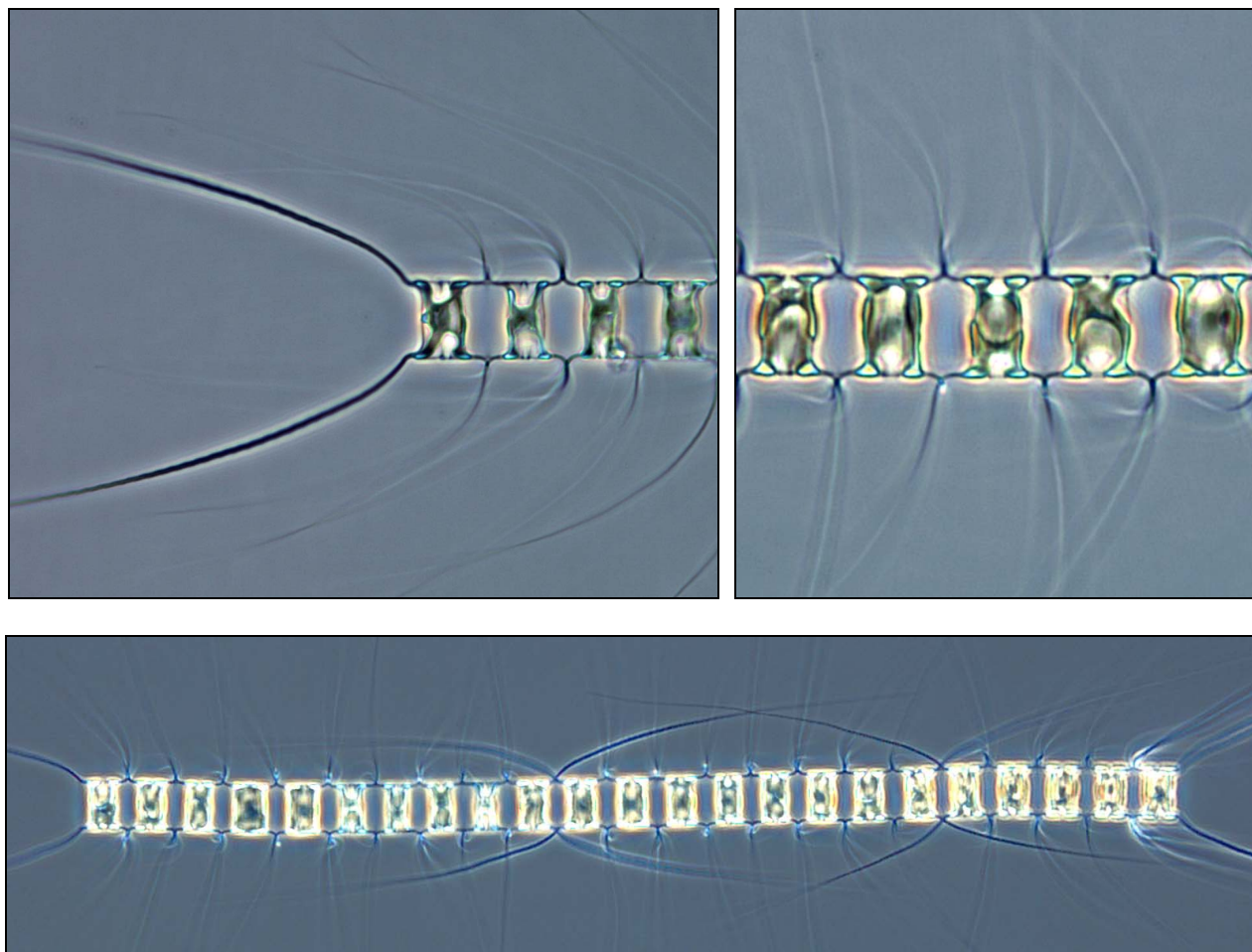
**Figure 1. Phase contrast light micrographs of the diatom *Chaetoceros concavicornis*. Upper left, vegetative colonies with numerous chloroplasts distributed both in the bodies of the cells, and in the hollow setae. Upper right, a colony with four auxospores, or zygotes, the product of sexual reproduction. Below, a colony in various stages of male gamete formation.**

CytoSense Evaluation. Our CytoSense scanning, in-line flow cytometer was specifically designed to study the size, shape, physiological and optical properties of phytoplankton colonies and individual cells within the colonies. It can be used both bench-top, and *in situ*. It streams near-real-time, multi-channel data on the size and optical properties of each particle as it flows past the sensors, creating a detailed scan of the variations in complexity of each parameter over the length of the particle. Our instrument contains a blue (488nm) laser, and sensors to measure forward scatter, side scatter, red, orange, yellow and green fluorescence, and curvature. We are using this instrument in two ways: (1) to analyze small, single cells, and (2) in our continuing investigations of the interactions between phytoplankton cell/colony size, morphology, small-scale turbulence, and optics.

We conducted a series of experiments to compare the results of analysis of small, single cells such as *Synechococcus* and *Synechocystis* (< 2µm Cyanobacteria) and *Dunaliella* (a ~ 6µm unicellular, eukaryotic green flagellate) when characterized by epifluorescence microscopy (both manual counts and image analysis) and CytoSense flow cytometry. The results are consistent (McFarland *et al.*, *in prep.*). We now turn our attention to the long, complex and beautiful colonies of diatoms that are of the greatest interest to us. CytoSense proves to be a powerful tool, and we have discovered that we can

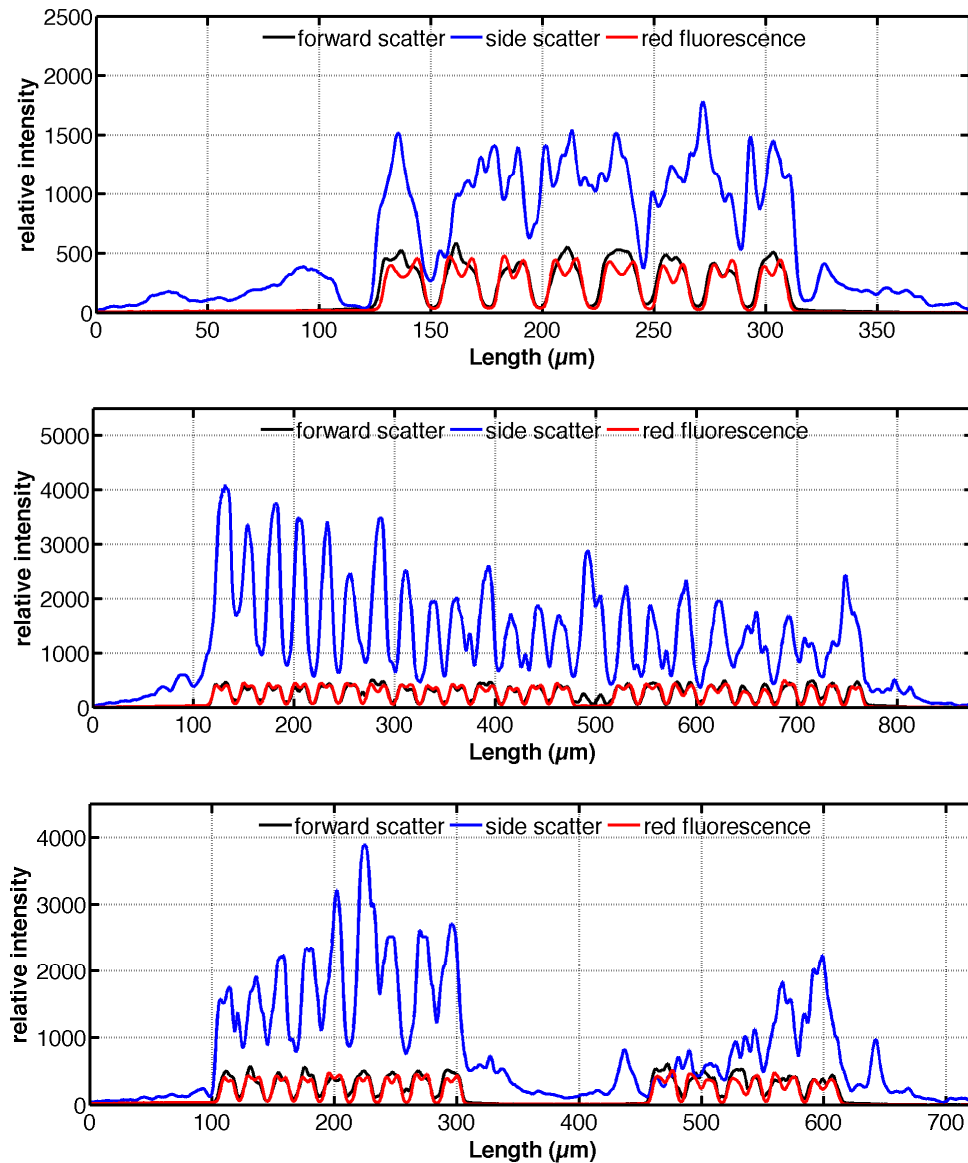


ask different questions by altering the trigger channel (e.g. chlorophyll fluorescence, forward scatter, side scatter), which initiates the start/stop ‘time of flight’ of a particle through the sensor system. For example, many species of the genus *Chaetoceros* have long siliceous terminal setae, which greatly increase the overall dimension of the particle (Figure 2). However, in taxa of the sub-genus *Hyalochaete*, they contain no chlorophyll. Thus, if chlorophyll fluorescence is used to trigger the collection of particle data, overall size will be underestimated. To collect data on the *Chaetoceros cf. distans* culture depicted in Figure 2, side scatter was used as the trigger channel (Figure 3).



**Figure 2. Phase contrast light micrographs of *Chaetoceros cf. distans*, depicting features, which can be resolved in CytoSense pulse profiles. Upper left, end of colony showing strongly differentiated and heavily silicified terminal setae. Upper right, five cells from center of colony, showing the single lobed chloroplast curled valve-to-valve inside each cell. Below, a long, complete colony in the process of dividing into three daughter colonies. Note the presence of differentiated terminal setae inside the chain. These cells are not fused to their neighbor, and will naturally separate.**

Many of the morphological and cytological features that can be seen in the microscope (e.g. Figure 2), are also depicted in CytoSense’s pulse profiles (Figure 3), and are discussed in that figure legend. Thus, CytoSense can be used to collect quantitative data in laboratory and field settings, which will allow us to pursue research on the impact of turbulence on chain forming diatoms, and on the optical properties of individual particles. We will use this information to (1) increase our understanding of the species-specific biology of the organisms, and (2) their impact on the IOPs of the water column.



**Figure 3.** Three CytoSense pulse profiles of *Chaetoceros cf. distans*, showing data from the forward scatter, side scatter and red (chlorophyll) fluorescence channels. The x-axis depicts particle length, as determined by time-of-flight past the sensor. The y-axis depicts the relative intensity of each channel, thus signal intensity cannot be compared among channels. Features of the pulse profiles can be compared to the morphology seen in the micrographs of Figure 2. Top panel, a 400 $\mu$ m colony. The terminal setae appear as ‘tails’ in the side scatter signature at each end, thus this is a complete colony. One that is broken in half (e.g. from turbulence) would be lacking one set of terminals. A strong side scatter signal comes from the numerous intercalary setae. Both the black (forward scatter) and red fluorescence (chlorophyll) signals can be used to determine that there are 8 cells in this colony. The double-humped red fluorescence in each cell is due to the single lobed chloroplast which is curled inside the cell, leading to a higher signal where overlapping occurs. The second panel depicts an approximately 900 $\mu$ m colony, with both terminal cells intact. At the 500 $\mu$ m point, there is a dead cell (no chlorophyll signal). The bottom panel depicts a colony, which is in the process of dividing into two daughter colonies. The new terminal setae appear in the side scatter signature between 300 and 450 $\mu$ m. The colonies have begun to slide apart, as evidenced by the gap in cells (forward scatter and chlorophyll fluorescence are both baselined).

## IMPACT/APPLICATIONS

Thin Layers of phytoplankton are an important feature of the coastal ocean. However, they don't exist in isolation – they are a component of the biological and hydrographic dynamics of the entire water column, and must be studied as such. Thin layers may simply contain an enhanced concentration of the phytoplankton community found throughout the water column, but frequently they contain a unique flora, with layers at different depths dominated by different taxa. Patterns exist at multiple scales. In addition to species-specific differences, we have demonstrated that *groups* of organisms (*e.g.* diatoms, dinoflagellates and picoplankton) can exhibit separate patterns of vertical distribution, thus different processes must regulate their dynamics. These relationships are not static: layers of motile organisms may migrate in and out of other structures. Thus, there may be many simultaneously occurring and interacting patterns, operating on multiple spatio-temporal scales.

Our LOCO data provides a wealth of information on the species-specific distribution of phytoplankton inside and outside of thin layers in Monterey Bay. When collaboratively combined with the physical, chemical, optical and acoustical data of our LOCO colleagues, we have a unique opportunity to further our knowledge of both the mechanisms of thin layer formation, maintenance and dissipation, and the biological, ecological and optical impacts of those layers.

Species-specific properties of phytoplankton such as size, shape, pigment composition, biomineralization and toxin production are known to play important ecological and oceanographic roles. However, the classical 'form and function' questions remain largely unanswered (Sournia 1982), and to my mind are amongst the most fascinating in biological oceanography. I am especially interested in the interactions between phytoplankton morphology (at both colony, and subcellular levels), physical mixing processes operating at the scale of the organism, and optics. Our CytoSense flow cytometer gives us a new, innovative tool with which to pursue the significance of particle variability with respect to biological/ecological questions, and also from the perspective of impact of species-specific properties of the phytoplankton on ocean optics. This instrument does not replace a microscope – its tremendous power lies in generating data to link IOPs to the highest quality, detailed microscopic images that we can obtain of the organisms themselves. We believe that CytoSense can quantify the optical properties of plankton in such a detailed way that it will both revolutionize our studies of phytoplankton ecology, and provide data critical to linking microscope-based studies of the species-specific properties of phytoplankton to the *in situ* inherent optical properties (IOPs) measured by our team.

## RELATED PROJECTS

We are working closely with Donaghay & Sullivan to link species-specific patterns of plankton distribution to physical and optical data. We are working with other members of the LOCO team to provide information on the phytoplankton to help them interpret their data sets.

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## PRESENTATIONS

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